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The Serological Differentiation of Hemolytic Streptococci of Human and Animal Origin.

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The Serological Differentiation of Hemolytic Streptococci of Human and Animal Origin

By P. R. EDWARDS

The hemolytic streptococci constitute a very large group of microorganisms which possess diverse physiological and biochemical characteristics. In discussing these forms it is desirable to define as exactly as possible the particular group to be described, otherwise confusion may result. Many of the contradictory results obtained in the study of streptococci are due to authors not describing clearly the organisms with which they worked. Hence, it is emphasized at this point that the present discussion deals only with the streptococci which produce a low acidity in glucose broth, which do not hydrolyze sodium hippurate, and which produce an active hemolysin in fluid mediums. In thus defining the cultures under consideration, the great group of streptococci commonly found in the udders of cows, in market milk, and in cheese, is excluded. The discussion is limited to those organisms which belong to groups A and C of Lancefield.

In previous publications (1–4) it was demonstrated that streptococci of human and animal origin can be differentiated with a fair degree of certainty by biochemical methods. The methods used to differentiate these organisms can be presented best in tabular form. The results obtained in a study of 183 cultures of animal origin and 120 cultures of human origin are given in Table 1.

TABLE 1. Biochemical Reactions of Streptococci.

Source	Number of Cultures	Lactose	Sorbitol	Trehalose	Reduction of Methylene Blue
Animal					
Str. equi.	17	—	—	—	—
Type A	159	+	+	—	—
Type B	7	±	—	+	+
Human	120	±	—	+	114— 6+

It will be noted that *Str. equi* was differentiated from all the other cultures studied by its failure to ferment either lactose, sorbitol, or trehalose. This organism was found only in strangles, or distemper, of the horse. Of the remaining animal cultures, 95.8 percent were classified as type A. They were distinguished from other cultures by their fermentation of sorbitol and their failure to attack trehalose. Type A is widely distributed among the laboratory and domestic animals. The remaining animal cultures were classified as type B. Cultures of this group resembled human cultures in their action on sorbitol and trehalose. However, all strains of this group reduced methylene blue, whereas only 5 percent of the human cultures reduced the dye.

These results have been confirmed in part by Plummer (5) and Lancefield (6). Plummer (5) examined 328 strains of human origin and found them uniformly trehalose positive and sorbitol negative. The results obtained with this large number of cultures and the 120 strains listed in Table 1 should establish beyond reasonable doubt that hemolytic streptococci of human origin ferment trehalose but do not attack sorbitol. Lancefield (6) worked with streptococci of human and animal origin as well as with high-acid-producing, sodium-hippurate-hydrolyzing strains of bovine and dairy origin. This investigator confirmed the fact that human strains attack trehalose and do not ferment sorbitol. *Str. equi* and the organisms here referred to as type A animal streptococci were studied by Lancefield. She confirmed the observation of the writer that these

organisms can be distinguished from human streptococci by their action on lactose, sorbitol and trehalose. No representatives of the organisms here referred to as type B were included in her work.

Lancefield (6) further observed that the hemolytic streptococci of human and animal origin can be distinguished by group precipitin tests. The present paper is a confirmation of the serological differentiation of Lancefield and an application of this method of study to the type B strains. While fermentation reactions offer a simple and practical method for the differentiation of *Str. equi* and the type A strains, the identity of the type B strains is left in some doubt because of the similarity of their fermentative reactions to those of the human streptococci. It is true that type B cultures all reduce methylene blue, but this is a quantitative test and some of the cultures recovered from human sources reduce the dye quite as actively as the type B strains. Additional evidence that they are distinct from streptococci of human origin will strengthen the conclusions previously published (1–4).

SOURCES AND CHARACTERISTICS OF CULTURES

The sources of the cultures are indicated briefly in Table 2. For a detailed description of the origin of the cultures and their biochemical characteristics the reader is referred to Kentucky Agricultural Experiment Station Bulletin 338 (1933).

TABLE 2. Sources and Characteristics of Cultures.

Origin of Cultures			Fermentation Reactions			Reduction of Methylene Blue	Precipitin Tests			
							Antisera for			
Strain	Source	Disease	Lactose	Sorbitol	Trehalose		Str. equi (D1)	Animal Type A (2)	Animal Type B (36)	Human (S23)
Streptococcus equi										
D1	Horse	Strangles	−	−	−	−	+	+	+	−
D2	Horse	Strangles	−	−	−	−	+	+	+	−
D3	Horse	Strangles	−	−	−	−	+	+	+	−
D10	Horse	Strangles	−	−	−	−	+	+	+	−
D11	Horse	Strangles	−	−	−	−	+	+	+	−
D12	Horse	Strangles	−	−	−	−	+	+	+	−
D15	Horse	Strangles	−	−	−	−	+	+	+	−
Type A. Animal Streptococci										
1	Horse	Chronic endometritis	+	+	−	−	+	+	+	−
2	Horse	Chronic endometritis	+	+	−	−	+	+	+	−
3	Horse	Chronic endometritis	+	+	−	−	+	+	+	−
8	Horse	Chronic endometritis	+	+	−	−	+	+	+	−
9	Horse	Chronic endometritis	+	+	−	−	+	+	+	−
10	Horse	Chronic endometritis	+	+	−	−	+	+	+	−
11	Horse	Chronic endometritis	+	+	−	−	+	+	+	−
12	Horse	Chronic endometritis	+	+	−	−	+	+	+	−
18	Horse	Chronic endometritis	+	+	−	−	+	+	+	−
26	Horse	Chronic endometritis	+	+	−	−	+	+	+	−
33	Horse	Chronic endometritis	+	+	−	−	+	+	+	−
35	Horse	Chronic endometritis	+	+	−	−	+	+	+	−
10116	Horse	Aborted fetus	+	+	−	−	+	+	+	−
F1	Horse	Aborted fetus	+	+	−	−	+	+	+	−

F2	Horse	Aborted fetus	+	+	—	—	+	+	+	—
F3	Horse	Septicemia	+	+	—	—	+	+	+	—
F4	Horse	Septicemia	+	+	—	—	+	+	+	—
F5	Horse	Septicemia	+	+	—	—	+	+	+	—
F9	Horse	Arthritis	+	+	—	—	+	+	+	—
F10	Horse	Arthritis	+	+	—	—	+	+	+	—
F11	Horse	Arthritis	+	+	—	—	+	+	+	—
F14	Horse	Rhinitis	+	+	—	—	+	+	+	—
F15	Horse	Rhinitis	+	+	—	—	+	+	+	—
F16	Horse	Rhinitis	+	+	—	—	+	+	+	—
17647	Horse	Wound infection	+	+	—	—	+	+	+	—
10986	Hog	Septicemia	+	+	—	—	+	+	+	—
22731	Hog	Septicemia	+	+	—	—	+	+	+	—
25273	Hog	Aborted fetus	+	+	—	—	+	+	+	—
31244	Hog	Abortion	+	+	—	—	+	+	+	—
611B	Guinea pig	Lymphadentis	+	+	—	—	+	+	+	—
Gp1F	Guinea pig	Lymphadentis	+	+	—	—	+	+	+	—
GPA	Guinea pig	Lymphadentis	+	+	—	—	+	+	+	—
GPB	Guinea pig	Lymphadentis	+	+	—	—	+	+	+	—
3962	Chicken	Slipped tendon	+	+	—	—	+	+	+	—
6554	Chicken	Slipped tendon	+	+	—	—	+	+	+	—
17090	Chicken	Slipped tendon	+	+	—	—	+	+	+	—
17098	Chicken	Slipped tendon	+	+	—	—	+	+	+	—
H	Chicken	Septicemia	+	+	—	—	+	+	+	—
3580	Fox	Pnuemonia	+	+	—	—	+	+	+	—
Clark	Rabbit	Septicemia	+	+	—	—	+	+	+	—
18783	Cow	Septicemia	+	+	—	—	+	+	+	—
25261	Cow	Abortion	+	+	—	—	+	+	+	—
25505	Cow	Septicemia	+	+	—	—	+	+	+	—
28117	Cow	Septicemia	+	+	—	—	+	+	+	—
126A	Cow	Metritis	+	+	—	—	+	+	+	—
E1	Cow	Mastitis	+	+	—	—	+	+	+	—
E2	Cow	Mastitis	+	+	—	—	+	+	+	—
E5	Cow	Mastitis	+	+	—	—	+	+	+	—
E6	Cow	Mastitis	+	+	—	—	+	+	+	—
E7	Cow	Mastitis	+	+	—	—	+	+	+	—
E8	Cow	Mastitis	+	+	—	—	+	+	+	—
E9	Cow	Mastitis	+	+	—	—	+	+	+	—
E10	Cow	Mastitis	+	+	—	—	+	+	+	—
E12	Cow	Mastitis	+	+	—	—	+	+	+	—
E13	Cow	Mastitis	+	+	—	—	+	+	+	—
E14	*	*	+	+	—	—	+	+	+	—
E15	*	*	+	+	—	—	+	+	+	—

TABLE 2—Continued.

Origin of Cultures			Fermentation Reactions			Reduction of Methylene Blue	Precipitin Tests			
							Antisera for			
Strain	Source	Disease	Lactose	Sorbitol	Trehalose		Str. equi (D1)	Animal Type A (2)	Animal Type B (36)	Human (S23)
Type B. Animal Streptococci										
4	Horse	Slight cervicitis	−	−	+	+	+	+	+	−
5	Horse	Slight cervicitis	+	−	+	+	+	+	+	−
36	Horse	None	−	−	+	+	+	+	+	−
55	Horse	None	−	−	+	+	+	+	+	−
F6	Horse	Abortion	−	−	+	+	+	+	+	−
126B	Cow	Metritis	−	−	+	+	+	+	+	−
25272	Hog	Abortion	−	−	+	+	+	+	+	−
H8	Human	None	+	−	+	+	+	+	+	−
H26	Human	Hemorrhagic smallpox	−	−	+	+	+	+	+	−
H91	Human	None	−	−	+	+	+	+	+	−
H112	Human	None	−	−	+	+	+	+	+	−
Human Streptococci										
S23	Human	Pneumonia	+	−	+	−	−	−	−	+
S43	Human	Pneumonia	+	−	+	−	−	−	−	+
NY5	Human	Scarlet fever	+	−	+	−	−	−	−	+
C203	Human	Scarlet fever	+	−	+	−	−	−	−	+
H3	Human	Mastoiditis	+	−	+	−	−	−	−	+
H4	Human	Mastoiditis	+	−	+	−	−	−	−	+
H5	Human	Mastoiditis	+	−	+	−	−	−	−	+
H11	Human	Meningitis	+	−	+	−	−	−	−	+
H28	Human	Scarlet fever	+	−	+	−	−	−	−	+
H34	Human	Scarlet fever	+	−	+	−	−	−	−	+
H36	Human	Measles	+	−	+	−	−	−	−	+
H39	Human	Puerperal sepsis	+	−	+	−	−	−	−	+

H40	Human	Puerperal sepsis	+	—	+	—	—	—	—	+
H42	Human	Tonsillitis	+	—	+	—	—	—	—	+
H46	Human	Erysipelas	+	—	+	—	—	—	—	+
H51	Human	Erysipelas	+	—	+	—	—	—	—	+
H56	Human	Pleuritis	—	—	+	—	—	—	—	+
H57	Human	Erysipelas	+	—	+	—	—	—	—	+
E41	Human	Sore throat	+	—	+	—	—	—	—	+
E42	Human	Sore throat	+	—	+	—	—	—	—	+
E44	Human	Otitis	+	—	+	—	—	—	—	+
E46	Human	Sore throat	+	—	+	—	—	—	—	+
E50	Human	Erysipelas	+	—	+	—	—	—	—	+
E53	Human	Erysipelas	+	—	+	—	—	—	—	+
E63	Human	Sore throat	+	—	+	—	—	—	—	+
E66	Human	Sore throat	+	—	+	—	—	—	—	+
E67	Human	Sore throat	+	—	+	—	—	—	—	+
E45	Cow	Mastitis	+	—	+	—	—	—	—	+
E55	Cow	Mastitis	+	—	+	—	—	—	—	+
E57	Cow	Mastitis	+	—	+	—	—	—	—	+
C	Cow	Mastitis	+	—	+	—	—	—	—	+
Gibbs I	Chicken	Bronchitis	—	—	+	—	—	—	—	+
Gibbs II	Chicken	Bronchitis	—	—	+	—	—	—	—	+
Gibbs III	Chicken	Bronchitis	—	—	+	—	—	—	—	+

* Cultures E14 and E15 were received as streptococci isolated from cases of septic sore throat in the Boston (1911) and Baltimore (1912) epidemics. Cultures of supposedly the same lineage were received from two other laboratories. The latter cultures were members of the human group.

METHODS

The methods used in studying fermentation and the reduction of methylene blue are those reported by Edwards (2). In performing the group precipitin tests the method of Lancefield (6) was used. In the preparation of antigens the organisms were centrifuged from 250cc. of an eighteen-hour broth culture and suspended in 5cc. of a physiological saline. Normal hydrochloric acid was added until the suspension was sufficiently acid to turn Congo-red paper blue. The acidified suspension was placed in a boiling water bath for ten minutes, removed and cooled rapidly. The organisms were centrifuged out and the supernatant fluid poured off and made neutral to litmus with sodium hydroxide. The liquid was cleared by centrifugation, and the water-clear supernatant fluid employed as antigen in the tests.

In the preparation of antisera, formalin-killed cultures were injected daily for seven days. This was followed by a seven day rest period, after which the daily injections were repeated. Satisfactory sera were obtained after two to four series of injections.

In performing the test, 0.2cc. of undiluted antiserum was used in each tube. Antigen was used in amounts of 0.4cc., 0.1cc., and 0.025cc. The total volume of the test was 0.6cc. Adequate serum and antigen controls were provided in all cases. The antigens were layered over the serums and the tests examined for ring formation after thirty minutes at room temperature. The tubes were then shaken, placed in the incubator at 37° for two hours and then read. A final reading was taken after storage in the icebox over night.

Positive tests gave marked ring formation and a heavy precipitate on incubation, while negative tests remained perfectly clear. No weak or doubtful tests were encountered; the results were clear-cut in all instances.

RESULTS

The results of the precipitin tests, the fermentation reactions, and the action of the cultures on methylene blue are re-

corded in Table 2. It will be noted that *Str. equi* and types A and B of the animal streptococci all cross precipitate. Four cultures recovered from human sources are included among the type B animal strains. These cultures resembled the type B strains both in serological and biochemical characters. The remainder of the human strains failed to precipitate with sera derived from animal cultures but reacted with the serum prepared from cultures of human origin. In addition, three cultures said to be derived from chickens and four cultures isolated from cows possessed the biochemical and serological characters of the human strains.

In addition to the serums listed in Table 2, serums were prepared with 2 type A strains, 3 type B strains, and one human strain. Also a serum prepared with a human strain by Dr. R. C. Lancefield was tested with a few of the antigens. Since all these serums gave results identical with those recorded, they were not included in the Table.

DISCUSSION

In the interpretation of the results reported here two facts must be kept in mind. First, only the low-acid-producing, non-sodium-hippurate hydrolyzing streptococci are considered in this work. These organisms are similar to those studied by Smith (7), Diernhofer (8), Seelemann and Hadenfeldt (9, 10), Hergesell (11), and Minett and Stableforth (12). All of these workers found that the animal strains in question resembled streptococci of human origin. The cultures studied here belong to the groups A and C of Lancefield (6). The division of the animal strains into two types, A and B, on the basis of fermentation reactions, should not be confused with the groups Lancefield established by serological methods. The biochemical types used here are the same as those used by Ogura (13) in the study of streptococci of horses. Since his types have been found to apply equally well to streptococci of other animals they have been retained. It is unfortunate that the groups of Lancefield and the types of Ogura are both designated by letters. *Str. equi,* types A and B of the animal streptococci all fall in group C of Lancefield. Lancefield's group A contains only human strains.

The second point to be kept in mind is that no type specificity is claimed for the precipitin tests presented here. These tests are only group specific. The antigens employed were crude HCl extracts of the streptococci. Lancefield (14, 15, 16) demonstrated two haptens in acid extracts of human streptococci. One, a protein, was type specific, the second was carbohydrate in nature and was present in extracts of hemolytic streptococci of human origin. It is upon this second, nonspecific antigen that the present differentiation is based. These group antigens, in all probability, correspond to the residue antigens of Zinsser and Parker (17) and Hitchcock (18, 19).

It is quite surprising that *Str. equi* and the types A and B animal streptococci possess a common antigen whereas their biochemical characteristics are quite diverse. *Str. equi* is not differentiated from the other animal cultures by the group tests employed. However, its biochemical properties set it apart from all the other cultures studied. Recently Evans (20) stated that *Str. equi* could be distinguished from other cultures by its behavior to "nascent" bacteriophage. Using this criterion she described several varieties of *Str. equi,* some of which fermented lactose. According to Evans' views these lactose fermenters must be regarded as *Str. equi* "on account of their ability to produce strangles in horses and their sensitiveness to four types of phage". The writer disagrees with these conclusions for several reasons. First Holth (21), Adsersen (22) and Ogura (13) have demonstrated that there is found constantly in strangles a particular type of streptococcus which ferments neither lactose, sorbitol, nor trehalose. These workers examined more than 250 cultures of these streptococci and found them absolutely uniform in their biochemical properties. Furthermore Zlatogoroff, Kandyba and Sadowsky (23) were able to reproduce the clinical manifestations of strangles only with streptococci possessing the properties attributed by Adsersen to *Str. equi.* In addition Ogura (24) and Zlatogoroff, Kandyba and Sadowsky (25) were able to immunize horses to strangles by using organisms having the characters attributed by Adsersen to *Str. equi.* Finally, Evans fails to take into account that

the mucous membranes of horses suffering with strangles may harbor other types of streptococci in addition to *Str. equi.* It has been emphasized by Edwards (2) and Dimock and Edwards (4, 26) that other streptococci do accompany *Str. equi* in strangles. Type A streptococci can be isolated from the exudate resulting from inflammation of the nasal or vaginal mucous membranes, regardless of the primary cause of the inflammation. These organisms may be found on the nasal mucous membranes in rhinitis and influenza and in the vaginal and uterine secretions following normal parturition in mares. Hence it is not surprising that lactose-fermenting streptococci have been found in strangles. However, in the light of our present knowledge of the disease, one is not justified in concluding that it is connected etiologically with streptococci other than those characterized as *Str. equi* in the present paper.

In contrast with *Str. equi* the type A animal streptococci are widely distributed thruout the various species of domestic animals. They are the organisms ordinarily encountered in hemolytic streptococcic infections of the domestic animals other than cows. The most common forms found in bovine infections are the hippurate-hydrolyzing strains, representatives of group B of Lancefield. That the group under discussion is occasionally present in bovine infections is attested by the numerous strains in Table 2 which were isolated from cattle. These organisms, when present in bovine mastitis, are often confused with septic sore throat streptococci. It has been demonstrated by the writer (3) that these organisms are really of animal origin and can be distinguished from the human cultures by their biochemical reactions. That their serological reactions also resemble those of the animal forms greatly strengthens the conclusions previously drawn and should remove all doubt of their identity. It is highly probable that organisms of this type do not cause human disease. Altho two cultures of this group, E14 and E15, were said to have been isolated from humans, their authenticity is extremely doubtful. Two other laboratories furnished cultures supposed to be lineal descendants of the

same stock as E14 and E15, all of which possessed all the characteristics of the human type.

Heretofore the type B streptococci were distinguished from the human cultures only by their greater ability to reduce methylene blue. The precipitin tests reveal that they have group antigens typical of the animal strains and that they are not serologically related to streptococci of human origin. The precipitin test is a much more satisfactory method of differentiating these strains than was formerly available. In a previous publication (2) it was noted that certain strains isolated from humans reduced methylene blue quite as actively as did the type B animal cultures. It is now revealed that these cultures possess the serological characters of the animal strains and that none of the cultures of the human type have the power to reduce methylene blue under the conditions of the experiments.

The occurrence of animal strains in human throats has been recorded by Plummer (5). These cultures were recovered from the throats of persons consuming raw milk and were thought to be of bovine origin. Lancefield and Hare (27) have recorded the occurrence of animal streptococci in the vaginal secretions of parturient women having a normal puerperium. As shown by Hare and Colebrook (28) most of these cultures were of the hippurate-hydrolyzing bovine type, but some corresponded in their biochemical and serological properties to the type B animal strains. These cultures had no connection with puerperal fever or any other clinical manifestation of disease in the human.

Three of the type B cultures isolated from humans were recovered from normal throats. There were very few colonies of hemolytic streptococci on the blood plates inoculated with throat swabs. These cultures were derived from the throats of inmates of a state institution where a search was being made for carriers of hemolytic streptococci. The inmates of this institution are supplied with raw milk, so that it is possible that the organisms were actually of bovine origin. The fact that these cultures were derived from apparently normal throats and that they were present in small numbers does not suggest

that they were the cause of human disease. The fourth type B culture isolated from the human was received from Dr. C. A. Behrens as one of the cultures recovered by Fisher (29) from hemorrhagic smallpox. The role of this type of streptococcus in human infections cannot be stated definitely at the present time. The type B animal strains in general are low in virulence and have been regarded as saprophytes. As stated by Dimock and Edwards (4) they may be found in normal animals, and when present in severe infections are usually associated with type A streptococci or *Str. equi*. When types A and B are both present in an infection the two types usually can be separated without difficulty. Blood plates streaked with the infective material yield two types of colonies. The type B colonies can be distinguished (from the type A colonies) by their larger size, dry, dull surface and narrower zones of hemolysis. In the writer's experience they never produce capsules, whereas the type A strains are pronounced capsule producers.

The human cultures form a distinct group having uniform biochemical and serological properties. These cultures correspond to group A of Lancefield's classification. They were largely derived from definite infections in humans but seven strains from animals are included in the group. Four of these latter strains were derived from the udders of cows, to which epidemics of septic sore throat had been traced. That their serological properties agree with those of cultures isolated from humans confirms the generally accepted opinion that epidemics of septic sore throat may be traced to the invasion of the udder of the cow by streptococci of human origin.

Three cultures said to be derived from the respiratory tract of chicks suffering with bronchitis are included in the group of human strains. These are the cultures described by Gibbs (30), who compared them with cultures, designated as *Str. pyogenes* and *Str. epidemicus*. The chicken strains, Gibbs stated, differed from the human cultures in that they were Gram negative, fermented raffinose and inulin, did not attack levulose, and were pathogenic for baby chicks. Dr. Gibbs' findings have not been confirmed. In the writer's hands the cultures proved to be

Gram positive cocci which ferment levulose and fail to ferment raffinose and inulin. It may be remarked in passing that the fermentation of inulin is a property foreign to the hemolytic streptococci, and the fermentation of this substance has been used to differentiate streptococci and pneumococci. Gibbs failed to take into consideration that there is no established agglutinative type representative of *Str. pyogenes* or *Str. epidemicus*. Agglutinative differences are not sufficient basis for the conclusion that the organisms represent a new and distinct type. Since the organisms studied here differ in so many respects from Gibbs' description, the question may be raised as to whether the organisms studied by the writer are the same streptococci designated by Gibbs as *Str. bronchitis*. All that can be said is that the organisms received as streptococci isolated from the respiratory tract of baby chicks resemble human streptococci by the criteria used here to differentiate human and animal strains.

Transplants of the three Gibbs strains were sent to Dr. W. S. Tillett, who tested their ability to dissolve human fibrin. Dr. Tillett has kindly permitted the writer to publish the results of his tests on these three strains. The culture designated Gibbs II dissolved fibrin regularly in each of seven different tests, acting in this respect like a typical human streptococcus. Gibbs I was negative in two tests and five times caused a slow dissolution of the fibrin. Gibbs III gave negative results in each of seven different tests. Unfortunately the results obtained by Dr. Tillett do not lend undivided support to the writer's conclusion that these cultures are of human origin. In this connection it is noteworthy that Plummer (5) isolated two cultures of streptococci from animals which were indistinguishable from human strains by the tests she employed. One of these organisms was isolated from a rabbit, the second from a horse.

CONCLUSIONS

1. Hemolytic streptococci of human and animal origin can be differentiated by precipitin tests in which acid extracts of the organisms are used as antigens.

2. The results of the precipitin tests are in agreement

with the conclusions previously reached by biochemical methods. The differentiation of streptococci by the methods previously recommended is thereby strengthened.

3. *Str. equi* and types A and B animal streptococci all fall into the same serological group by the tests used. At the present time these organisms can be differentiated only by biochemical methods.

4. The type A animal streptococci are probably purely animal parasites. These organisms have not been found in human disease.

5. Four strains isolated from human sources were found to be members of the type B animal streptococci. Three of these cultures were isolated from apparently normal throats of consumers of raw milk. No definite statement concerning the role of the type B strains in the production of human disease can be made at present.

6. Seven strains said to have been isolated from animals were found to be members of the human type. Four of these were isolated from the udders of cows to which epidemics of septic sore throat had been traced. The remaining three cultures were said to be derived from the respiratory tract of chicks. These cultures possessed characteristics at variance with the original description of the cultures from chicks.

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